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Management of Cucumber Mosaic Virus (CMV) Disease in Chilli through Biotic Defense Inducers

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A B S T R A C T

Different biotic defense inducers such as, red seaweed extract (*Kappaphycus alvarezii*), plant extracts (*Mirabilis jalapa* L. and *Bougainvillea spectabilis* Willd.), biocontrol agents (*Trichoderma harzianum* and *Pseudomonas fluorescens*) and other defense inducing biomolecules *viz.*, synthetic nucleoside, chitosan and virex-H were tested for their efficacy on Cucumber mosaic virus (CMV) infection in Chilli *var*. Pusa Jwala during *Kharif*2016-17and *Kharif* 2017-18under field conditions. Results showed that a significantly less PDI (35.29 %) and AUDPC (1238.33) was observed in plants treated with Chitosan (0.1%) with enhanced plant height (49.19 cm), number of branches (19.52 no.), number of fruits per plant (187.87 no.), individual fruit weight (2.44 g), fresh fruit yield (483.56 g.plant^{-1}), fresh weight (4.59 t.ha^{-1}), dry weight (1.38 t.ha^{-1}). Significant differences among the treatments by reducing the CMV disease with increased growth and yield was observed and is found superior over the untreated control.

Introduction

Chilli (*Capsicum annuum* L.) is an important spice grown for its fruits, which are used in green as well as ripe dried form for its pungency. Chilli belongs to the genus Capsicum, family Solanaceae. It has originated in Mexico, Southern Peru and Bolivia (Villalon, 1981). India is the largest producer of chilli in the world with a production of 1492.14 MT. The average national productivity of chilli in India is 1.92MT/ha (IHD, 2015). Major chilli growing states in India are Andhra Pradesh, Maharashtra, Karnataka and Tamil Nadu which together contribute about 75 per cent of the total area (Bhadramurthy *et al.*, 2009).

Chilli is attacked by more than 30 different viruses throughout the world and the estimated collective yield losses ranging from 15 to 50 per cent (Agranovsky, 1993; Green and Kim, 1991). CMV is found to be the most bounteous virus on chilli, especially in tropic and semi-tropic regions, which provide ideal conditions for the virus perpetuation and their vectors (Biswas *et al.*, 2013; Myti *et al.*, 2014). Infection of CMV in chilli often leads to losses of 10–20 per cent in yield and even if harvested. In severe infections, yield losses

may reach up to 60 per cent if plants are infected at an early stage (Ong *et al.,* 1979), with the plants producing none or only very few fruits, which are small in size (Jones *et al.,* 1991).

CMV exhibit complex symptoms *viz*., mosaic, mottle, leaf distortion, vein chlorosis and stunting (Plate 1) causing considerable loss in yield and plant vigor (Rashid *et al.,* 2007). CMV is easily transmitted by mechanical inoculation as well as by more than 80 species of aphids in non-persistent manner (Palukaitis and Garcia-Arenal, 2003). The coat protein (CP) of CMV is a primary determinant of aphid transmission (Chen and Francki, 1990). Seed transmission of CMV has been accounted for 0 to 100 per cent in different host species, including weed species (Neergaard, 1977). Weed hosts function as a repository for the virus and serve as primary source of inoculum for the development of disease epidemics (Grube *et al.,* 2000).

In order to conquer production losses, different approaches are generally been adopted for the management of plant viral diseases. Use of inducible defense mechanisms against viruses has been shown to be effective in many plant species (Murphy, 2006). Control of virus disease is only can be done by controlling its vector using insecticides. Besides not being effective, it also has negative impact on human health and environment. Incorrect use of insecticides in both types and doses often causes problems because they can increase production costs and can leave residuals on production (Astuti*et al.,* 2013). Utilization of non-hazardous materials such as the use of plants extracts, seaweed extracts and bio-control agents and their effectiveness are being investigated. Several researcher reports indicated, plant extracts and other defense inducers could be used to control various causes of plant diseases and are able to induce plant resistance (Madhusudhan *et al.,* 2005). Therefore, the present study was conducted to evaluate a few biotic inducers to induce systemic resistance and safe means of controlling virus infection in chilli under open field conditions.

Materials and Methods

A field experiment was carried out during two growing *Kharif* seasons of 2016-17 and 2017-18, at MRS, Hebbal, Bengaluru in order to investigate the impact of foliar application of various biotic inducers on CMV incidence, growth and yield of chilli in *var*. PusaJwala. The chilli seedlings were primed with different biotic inducers separately and planted in the field with a spacing of 60 × 45 cm. Cultural practices, pest and disease were taken care of by following package of practices of UAS, GKVK, Bengaluru.

Biotic inducers used for management of CMV disease

The experiment was carried out by using different treatments such as, red seaweed extract (*Kappaphycus alvarezii*), plant extracts, biocontrol agents and other defence inducing bio-molecules (Table 1 and Plate 1).

Preparation of crude extracts of botanicals

Crude extracts were prepared from the leaves of *M. jalapa* L. (Sanjemallige) and *B. spectabilis* Willd. (Paper flower). Hundred grams of leaves was blended in a grinder containing 100mL sterilized distilled water at a ratio of 1:1 (weight/volume). The sample was spun at low speed for 10-15 min., the blended material was squeezed through a sterile muslin cloth to get a crude liquid extract and it was filtered through Whatman No. 1 filter paper. The filtrate was collected in sterilized glass tubes and the tubes were sealed under aseptic condition. The water extract so

obtained was used within 6 hours of preparation (Thippeswamy, 2010).

Culture filtrates of biological control agents

Culture filtrates of *T. harzianum* and *P. fluorescens* was obtained by growing on potato dextrose broth (PDB) and nutrient broth (NB). Conical flasks of half litre capacity containing 250 mL PDB and NB were inoculated with 5 mm mycelial plugs of 7 days old culture grown on PDA and loopful culture of bacteria grown on nutrient agar respectively. Culture flasks were placed on an orbital shaker (150 rotations.min^{-1}) during the first week. After inoculation, culture flasks were checked visually and flasks showing only pure growth of their respective isolate after 35days of incubation were taken to collect culture filtrates. The liquid medium was filtered through oven dried Whatman No.42 filter paper to separate fungal mycelium. Filtrate containing cell suspension was collected and was considered as 100per cent concentration (Vishwanath and Kolte, 1997).

Evaluation of biotic defense inducers

Foliar application of biotic inducers was done at 15 days intervals starting from a week after transplanting in the field. The observations for disease incidence and growth parameters were recorded at before initiation of first spray and 15 days after each spray and the data were analyzed statistically. The per cent disease inhibition over control was calculated by using the formula given by Vincent (1927).

$$\text{Disease inhibition (\%)} = \frac{(C-T)}{C} \times 100$$

Where,
C = Per cent disease in untreated control
T = Per cent disease in treatment

$$\text{Per cent yield increase over untreated} = \frac{Yt - Yc}{Yt} \times 100$$

Where, Yt- Yield of treated plant
Yc- yield of untreated control plant

Area under disease progress curve (AUDPC)

The Area under disease progressive curve was calculated by the trapezoidal integration of the percent disease index (PDI) over time for each treatmentaccording to Campbell and Madden (1990).

$$AUDPC = \sum_{i=1}^{n-1} \frac{(y_i + y_{i+1})}{2} \times (t_{i+1} - t_i)$$

where,

n: Number of assessment;
y: Percent disease index (PDI)
(t_{i+1}-t_i) is duration between two consecutive assessments

The disease assessments over specific periods of time interval *viz*., 30, 45 and 60 DAS was recorded during the experiments and interpreted according to the above mentioned formula. The AUDPC of CMV for each treatment was calculated and the data was analysed statistically.

Statistical analysis

All treatments were replicated thrice and organized in a randomized complete block design (RCBD). Data acquired from the present investigation was subjected to ANOVA (Sheoran *et al.,* 1998). Significance between the treatments were calculated according to Duncan's Multiple Range Test at p=0.05.

Results and Discussion

Effect of different treatments on systemic protection against CMV in chilli

In the present study, an attempt has been made to control CMV by using certain biotic defense inducers during *Kharif*2016-17and*Kharif*2017-18 under field conditions (Plate 2). The antiviral activity of different biotic inducers was assessed for their effect based on PDI and AUDPC. Plants exhibited different kind of symptoms includes mosaic, leaf distortion, rat tailling, and dwarfing (Plate 3) under field conditions.

At 65 DAT, chilli plants treated with chitosan (0.1%) showed less PDI of 29.83 and 36.64 per cent respectively in *Kharif*2016-17 *and Kharif* 2017-18, followed by *K. alvarezii* (30.35 and 39.48 %) and *P. fluorescens* (31.10 and 41.04 %) compared to control (49.07 and 62.06 %) (Table 2, Fig. 1). In addition, PDI was directly correlated with the AUDPC, chitosan treated plants showed AUDPC of 1162.35 and 1314.30 followed by *K. alvarezii* (1098.68 and 1425.55), *P. fluorescens* (1133.33 and 1584.28). Whereas, untreated control showed high AUDPC of 1768.84 and 2216.79 in *Kharif* 2016-17and *Kharif* 2017-18. The present investigations indicated that, there was a significant reduction in the severity of CMV disease in chilli treated with different biotic inducers compared to that of the untreated control.

The effectiveness of chitosan application for viral disease management under field condition has been reported by several workers (Hadrami *et al.,* 2010; Compant *et al.,* 2010; Noiket *et al.,* 2014). Chitosan treatment caused inhibition of tomato yellow leaf curl disease symptoms in tomato (Noiket *et al.,* 2014). The field experiments conducted for the management of CMV in gherkins using seaweed extracts during *Kharif* and *rabi* 2016 revealed that *K. alvarezii-1* (0.4%) recorded less mean PDI of 16.65 and 16.06 followed by *Halymenia durvillae* (1%) with mean PDI (18.34 and 18.98) compared to control with mean PDI (31.77 and 31.96) respectively (Venkatesh, 2016). Pushpa *et al.,* (2018) reported the delay in appearance of PRSV symptoms in papaya plants treated with *K. alvarezii* (0.4%) and produced more number of fruits (average 30 no's per plant) compared to untreated control (average 15 no's per plant). Similarly, biocontrol efficacy of *P. fluorescens* against CMV, tomato mottle virus and tomato spotted wilt virus (TSWV) in tomato under field conditions was observed by Murphy *et al.,* (2000) and Kandan *et al.,* (2003).

Chitosan induced resistance is may be due to increased activity chitinase enzyme, chitosan may inhibit virus replication, cell-to-cell movement and also activate receptor like kinases (RLK's) which induce systemic resistance (ISR) in the plants (Deni-Firmansyah *et al.,* 2017). The increased chitinase activity might have been prevented the damage caused by viral pathogen and, thus, increased the per cent disease control in chitosan treated plants. Synthesis and accumulation of PR proteins have been reported to play an important role in plant defence mechanisms. Chitinases, which are classified under PR-3 have been reported to associate with resistance in plants against pests and diseases (Maurhofer *et al.,* 1994; Van-Loon, 1997).

The PGPR, a biocontrol agent induce ISR by activating jasmonate and ethylene signalling pathways systemically in plants and these hormones stimulate the defence responses in host plants against a variety of plant pathogens (Deni-Firmansyah *et al.,* 2017). Rhizobacterial treated tomato plants showed to induce phenylalanine ammonia lyase (PAL), peroxidase (PO), Chitinase and polyphenol

oxidase (PPO). PAL activity during plant - pathogen and plant - pest interactions (Harish, 2005) and is known to play an important role in the biosynthesis of various defense chemicals in phenyl propanoid metabolism (Daayf *et al.,* 1997). Cinammic acid, the product of PAL, is directly linked to cell lignifications processes and the highest levels of PAL activity usually occur about one day after initial infection of pathogen (Podile and Laxmi, 1998)**.**

The molecular details on the effect of *K. alvarezii* extract in delaying CMV symptoms remain to be explored. However, it is likely that the sulphated galactans of *K. alvarezii* may activate plant's immune responses similarly as microbial elicitors *viz.,* bacterial peptidoglycans, flagellin, lipopolysaccharides and chitin of fungal cell wall that elicit MAMPs immune response (Boller and Felix, 2009; Macho and Zipfel, 2014). More recently, the red seaweed *Schyzimenia binderi* derived oligo-sulphated-galactan, poly-Ga, has been shown to induce long-term protection against TMV in tobacco plants (Vera *et al.,* 2011). It is possible that the sulphated oligosugars or any unknown chemical compounds present in *K. alvarezii* extract may mimic as MAMP elicitors leading to activation of plant's immune response, however further studies in this direction are essential for detailed understanding of their molecular mechanisms.

Plant growth promotional potential of different biotic inducers

The effect of different biotic inducers on growth and yield parameters of chilli was studied under field conditions. Chilli plants treated with *P. fluorescens* (0.6%) showed significantly increased plant height (51.42cm), number of branches (22.36) and less number of days for initial flowering (32.12) in addition to suppression of CMV disease, followed by chitosan (0.1%) and *K. alvarezii*(0.4%) (Table 3, Fig. 2).However compared to other treatments, treatments *viz.,* Virex-H and extract of *Mirabilis jalapa* L. (sanjemallige) resulted in significantly reduced growth.

Table.1 List of treatments used against CMV disease in chilli

Treatments		Dosage (%)
T1	Red seaweed (*Kappaphycus alvarezii* (Doty) Doty ex P.C.Silva) extract	0.4
T2	Synthetic nucleoside	0.25
T3	Extract of *Mirabilis jalapa* L. (Sanjemallige)	0.5
T4	Extract of *Bougainvillea spectabilis*Willd. (Paper flower)	0.5
T5	Fungal bio-control agent (*Trichoderma harzianum*)	0.6
T6	Bacterial bio-control agent (*Pseudomonas fluorescens*)	0.6
T7	Chitosan	0.1
T8	Virex-H	0.3
T9	Dimethoate 30per cent EC	0.2
T10	Untreated control	

Table.2 Effect of biotic inducers on incidence of CMV disease during *Kharif*-2016-17 and 2017-18 under field conditions

Treatments		*Kharif*- 2016-17					*Kharif*- 2017-18				
		PDI @ 30 DAT	PDI @ 45 DAT	PDI @ 60 DAT	AUDPC	DOC (%) @ 60 DAT	PDI @ 30 DAT	PDI @ 45 DAT	PDI @ 60 DAT	AUDPC	DOC (%) @ 60 DAT
T1	*K. alvarezii* (Doty) Doty ex P.C.Silva) @ 0.4%	21.30^{de}	26.12^{gf}	30.35^{f}	1098.68	38.15	28.62^{f}	32.36^{f}	39.48^{g}	1425.55	36.38
T2	Synthetic nucleoside @ 0.25%	23.83^{c}	32.45^{d}	35.38^{d}	1288.26	27.90	30.27^{e}	41.29^{c}	45.50^{cd}	1641.71	26.68
T3	*Mirabilis jalapa* L. @ 0.5%	20.02^{e}	30.00^{e}	31.37^{e}	1135.60	36.07	27.61^{g}	41.45^{c}	43.81^{de}	1571.60	29.41
T4	*B. spectabilis*Willd. @ 0.5%	21.32^{de}	26.37^{gf}	31.80^{e}	1113.67	35.19	30.59^{e}	37.89^{d}	46.19^{c}	1603.03	25.57
T5	*T. harzianum* @ 0.6%	20.10^{e}	26.10^{gf}	31.49^{e}	1079.94	35.83	27.11^{g}	35.26^{e}	42.99^{ef}	1461.13	30.73
T6	*P. fluorescens* @ 0.6%	22.83^{cd}	25.77^{g}	31.10^{e}	1133.33	36.62	32.53^{d}	36.31^{de}	41.04^{fg}	1584.28	33.87
T7	Chitosan @ 0.1%	23.94^{c}	26.67^{f}	29.83^{f}	1162.35	39.21	25.40^{h}	31.20^{f}	36.64^{h}	1314.30	40.96
T8	Virex-H @ 0.3%	34.55^{a}	42.14^{b}	47.80^{b}	1767.87	2.59	37.55^{b}	45.88^{b}	52.60^{b}	1927.58	15.24
T9	Dimethoate 30% EC @ 0.2%	30.02^{b}	37.27^{c}	45.63^{c}	1576.81	7.01	33.79^{c}	42.02^{c}	51.99^{b}	1780.45	16.23
T10	Untreated control	33.44^{a}	43.23^{a}	49.07^{a}	1768.84	0.00	41.77^{a}	54.10^{a}	62.06^{a}	2216.79	0.00
SEm±		0.498	0.146	0.176	--	--	0.233	0.465	0.520	--	--
CD @ 5%		1.479	0.435	0.522	--	--	0.693	1.383	1.544	--	--
CV %		3.430	0.801	0.836	--	--	1.281	2.027	1.947	--	--

***DOS:** 20-08-2017; **DOT:** 28-09-2017

PDI: Per cent disease incidence; **DAT:** Days after transplanting; **AUDPC:** Area under disease progress curve; **DOC:** Decrease over control; In each column, means followed by the same letter are not significantly different at p=0.05 according to Duncan's Multiple Range Test.

Table.3 Effect of biotic inducers on growth parameters of chilli during *Kharif* 2016-17 and 2017-18 under field conditions

Treatments		*Kharif*- 2016-17			*Kharif*- 2017-18		
		Plant height (cm)	Number of branches (no.)	Days to initiation of flowering	Plant height (cm)	Number of branches (no.)	Days to initiation of flowering
T1	*K. alvarezii* (Doty) Doty ex P.C.Silva) @ 0.4%	46.50 ^{d}	18.75 ^{b}	37.44 ^{g}	47.20 ^{c}	19.40 ^{b}	37.86 ^{g}
T2	Synthetic nucleoside @ 0.25%	45.52 ^{e}	12.54 ^{d}	40.56 ^{e}	46.13 ^{d}	12.96 ^{d}	41.19 ^{e}
T3	*Mirabilis jalapa* L. @ 0.5%	40.77 ^{g}	9.56 ^{f}	43.35 ^{c}	41.24 ^{f}	10.23 ^{f}	43.85 ^{c}
T4	*Bougainvillea spectabilis*Willd. @ 0.5%	42.36 ^{f}	11.45 ^{e}	42.00 ^{d}	42.89 ^{e}	11.36 ^{e}	42.15 ^{d}
T5	*Trichodermaharzianum* @ 0.6%	48.56 ^{c}	16.35 ^{c}	39.54 ^{f}	49.23 ^{b}	16.42 ^{c}	40.02 ^{f}
T6	*Pseudomonas fluorescens* @ 0.6%	51.42 ^{a}	22.36 ^{a}	32.12 ^{i}	52.14 ^{a}	22.15 ^{a}	32.45 ^{i}
T7	Chitosan @ 0.1%	49.25 ^{b}	19.24 ^{b}	35.24 ^{h}	49.13 ^{b}	19.80 ^{b}	35.36 ^{h}
T8	Virex-H @ 0.3%	39.35 ^{h}	8.90 ^{g}	45.23 ^{b}	39.86 ^{g}	9.70 ^{gf}	45.75 ^{b}
T9	Dimethoate 30% EC @ 0.2%	46.23 ^{d}	12.75 ^{d}	40.25 ^{e}	46.53 ^{cd}	13.40 ^{d}	40.90 ^{e}
T10	Untreated control	37.45 ^{i}	8.65 ^{g}	46.30 ^{a}	36.70 ^{h}	9.14 ^{g}	46.78 ^{a}
SEm±		0.092	0.121	0.085	0.179	0.16	0.193
CD @ 5%		0.273	0.359	0.253	0.532	0.476	0.574
CV %		0.356	1.49	0.367	0.688	1.92	0.823

*DOS: 20-08-2017; DOT: 28-09-2017; In each column, means followed by the same letter are not significantly different at p=0.05 according to Duncan's Multiple Range Test.

Table.4 Effect of biotic inducers on yield parameters of chilli during *Kharif* 2016-17 and 2017-18 under field conditions

Treatments	*Kharif*- 2016-17						*Kharif*- 2017-18					
	Number of fruits per plant	Individual fruit weight (g)	Fresh fruit yield (g/plant)	Fresh weight ($t.ha^{-1}$)	Dry weight ($t.ha^{-1}$)	IOC (%)	Number of fruits per plant	Individual fruit weight (g)	Fresh fruit yield (g/plant)	Fresh weight ($t.ha^{-1}$)	Dry weight ($t.ha^{-1}$)	IOC (%)
T1	178.25 ^{b}	2.48 ^{a}	475.41 ^{b}	4.74 ^{ab}	1.42 ^{ab}	16.67	169.35 ^{b}	2.21 ^{ab}	459.23 ^{b}	4.13 ^{ab}	1.24 ^{ab}	21.55
T2	159.53 ^{d}	2.35 ^{a}	448.60 ^{d}	4.57 ^{ab}	1.37 ^{ab}	13.57	150.03 ^{d}	2.13 ^{ab}	434.20 ^{c}	3.98 ^{abc}	1.19 ^{abc}	18.59
T3	122.98 ^{h}	2.01 ^{a}	429.42 ^{h}	4.02 ^{b}	1.21 ^{b}	1.74	113.40 ^{h}	1.83^{ab}	413.56 ^{h}	3.41 ^{cd}	1.02 ^{cd}	4.99
T4	137.25 ^{g}	2.13 ^{a}	431.21 ^{g}	4.23 ^{ab}	1.27 ^{ab}	6.62	127.80 ^{g}	1.86 ^{ab}	415.80 ^{g}	3.63 ^{bcd}	1.09 ^{bcd}	10.74
T5	142.50 ^{f}	2.20 ^{a}	435.54 ^{f}	4.29 ^{ab}	1.29 ^{ab}	7.93	133.13 ^{f}	1.93^{ab}	421.21 ^{f}	3.70 ^{abcd}	1.11 ^{abcd}	12.43
T6	162.80 ^{c}	2.42 ^{a}	456.24 ^{c}	4.68 ^{ab}	1.40 ^{ab}	15.60	152.54 ^{c}	2.18 ^{ab}	430.36 ^{d}	3.97 ^{abc}	1.19 ^{abc}	18.39
T7	192.70 ^{a}	2.56 ^{a}	490.86 ^{a}	4.89 ^{a}	1.47 ^{a}	19.22	183.04 ^{a}	2.32 ^{a}	476.25 ^{a}	4.29 ^{a}	1.29 ^{a}	24.48
T8	120.20 ^{i}	1.98 ^{a}	426.12 ^{i}	3.98 ^{b}	1.19 ^{b}	0.75	110.50 ^{i}	1.73 ^{b}	410.68 ^{i}	3.38 ^{cd}	1.01 ^{cd}	4.14
T9	143.60 ^{e}	2.26 ^{a}	436.58 ^{e}	4.36 ^{ab}	1.31 ^{ab}	9.40	134.20 ^{e}	2.12^{ab}	422.34 ^{e}	3.77 ^{abcd}	1.13 ^{abcd}	14.06
T10	112.36 ^{j}	1.90 ^{a}	425.36 ^{j}	3.95 ^{b}	1.19 ^{b}	0.00	103.40 ^{j}	1.71 ^{b}	409.53 ^{j}	3.24 ^{d}	0.97 ^{d}	0.00
SEm±	0.151	0.154	0.127	0.172	0.052	--	0.141	0.115	0.110	0.144	0.043	--
CD @ 5%	0.447	0.457	0.377	0.511	0.153	--	0.418	0.341	0.326	0.429	0.129	--
CV %	0.177	11.949	0.049	6.811	6.811	--	0.177	9.940	0.044	6.669	6.669	--

*DOS: 20-08-2017; DOT: 28-09-2017

IOC: Increase over control; In each column, means followed by the same letter are not significantly different at p=0.05 according to Duncan's Multiple Range Test

Table.5 Effect of biotic defense inducers on CMV disease incidence, growth and yield parameters of chilli under field conditions*

Treatments	PDI @ 30 DAT	PDI @ 45 DAT	PDI @ 60 DAT	Plant height (cm)	Number of branches (no.)	Days to initiation of flowering	Number of fruits per plant	Individual fruit weight(g)	Fresh fruit yield (g.plant^{-1})	Fresh weight (t.ha^{-1})	Dry weight (t.ha^{-1})
T1	23.35 ^{g}	28.66 ^{h}	33.50 ^{h}	46.85 ^{d}	19.08 ^{c}	37.65 ^{g}	173.80 ^{b}	2.35^{ab}	467.32 ^{b}	4.44^{ab}	1.33 ^{a}
T2	28.24 ^{d}	31.49^{gf}	35.44 ^{g}	45.83 ^{f}	12.75 ^{f}	40.88 ^{e}	154.78 ^{d}	2.24^{abc}	441.40 ^{d}	4.28 ^{abc}	1.28^{ab}
T3	23.82 ^{g}	35.73 ^{e}	37.59 ^{f}	41.01 ^{h}	9.90 ^{h}	43.60 ^{c}	118.19 ^{h}	1.92 ^{de}	421.49 ^{h}	3.72 ^{de}	1.11 ^{de}
T4	25.96 ^{f}	32.13 ^{f}	39.00 ^{e}	42.63 ^{g}	11.41 ^{g}	42.08 ^{d}	132.53 ^{g}	2.00^{cde}	423.51 ^{g}	3.93 ^{cde}	1.18^{cde}
T5	23.61 ^{g}	30.68 ^{g}	37.24 ^{f}	48.90 ^{c}	16.39 ^{d}	39.78 ^{f}	137.82 ^{f}	2.07^{bcde}	428.38 ^{f}	4.00^{cde}	1.20^{bcd}
T6	27.05 ^{e}	36.87 ^{d}	40.44 ^{d}	51.78 ^{a}	22.26 ^{a}	32.29 ^{i}	157.67 ^{c}	2.30^{ab}	443.30 ^{c}	4.33 ^{abc}	1.30^{ab}
T7	25.73 ^{f}	29.07 ^{h}	35.29 ^{g}	49.19 ^{b}	19.52 ^{b}	35.30 ^{h}	187.87 ^{a}	2.44 ^{a}	483.56 ^{a}	4.59 ^{a}	1.38 ^{a}
T8	36.05 ^{b}	44.01 ^{b}	50.20 ^{b}	39.61 ^{i}	9.30 ^{i}	45.49 ^{b}	115.35 ^{i}	1.86 ^{e}	418.40 ^{i}	3.68 ^{de}	1.10 ^{de}
T9	31.91 ^{c}	39.65 ^{c}	48.81 ^{c}	46.38 ^{e}	13.08 ^{e}	40.58 ^{e}	138.90 ^{e}	2.19^{abcd}	429.46 ^{e}	4.07 ^{bcd}	1.22^{bc}
T10	37.61 ^{a}	48.67 ^{a}	55.57 ^{a}	37.08 ^{j}	8.90 ^{j}	46.54 ^{a}	107.88 ^{j}	1.81 ^{e}	417.45 ^{j}	3.60 ^{e}	1.08 ^{e}
SEm±	0.546	0.686	0.764	0.137	0.119	0.103	0.126	0.086	0.779	0.101	0.030
CD @ 5%	1.554	1.955	2.177	0.391	0.340	0.292	0.359	0.246	2.219	0.288	0.086
CV %	4.717	4.711	4.533	0.749	2.051	0.622	0.216	10.006	0.436	6.090	6.090

*Pooled analysis; In each column, means followed by the same letter are not significantly different at p=0.05 according to Duncan's Multiple Range Test.

Plate.1 Different biotic defense inducers used for the management of CMV disease
T1- Red seaweed (*Kappaphycus alvarezii* (Doty) Doty ex P.C. Silva) extract (LBD 3); **T2-** Synthetic nucleoside (SEVI); **T3-** Extract of *Mirabilis jalapa* L. (Sanjemallige); **T4-** Extract of *Bougainvillea spectabilis* Willd. (Paper flower); **T5-** Fungal bio-control agent (*Trichoderma harzianum*); **T6-** Bacterial bio-control agent (*Pseudomonas fluorescens*); **T7-** Chitosan; **T8-** Virex-H; **T9**- Dimethoate 30% EC

Plate.2 Field view for the management of CMV disease in chilli using different biotic inducers at MRS, Hebbal, Bengaluru

Plate.3 Symptoms of CMV on naturally infected chilli plants

Fig.1 Effect of biotic inducers on the incidence of the CMV disease during *Kharif* 2016-17 & 2017-18 under field conditions

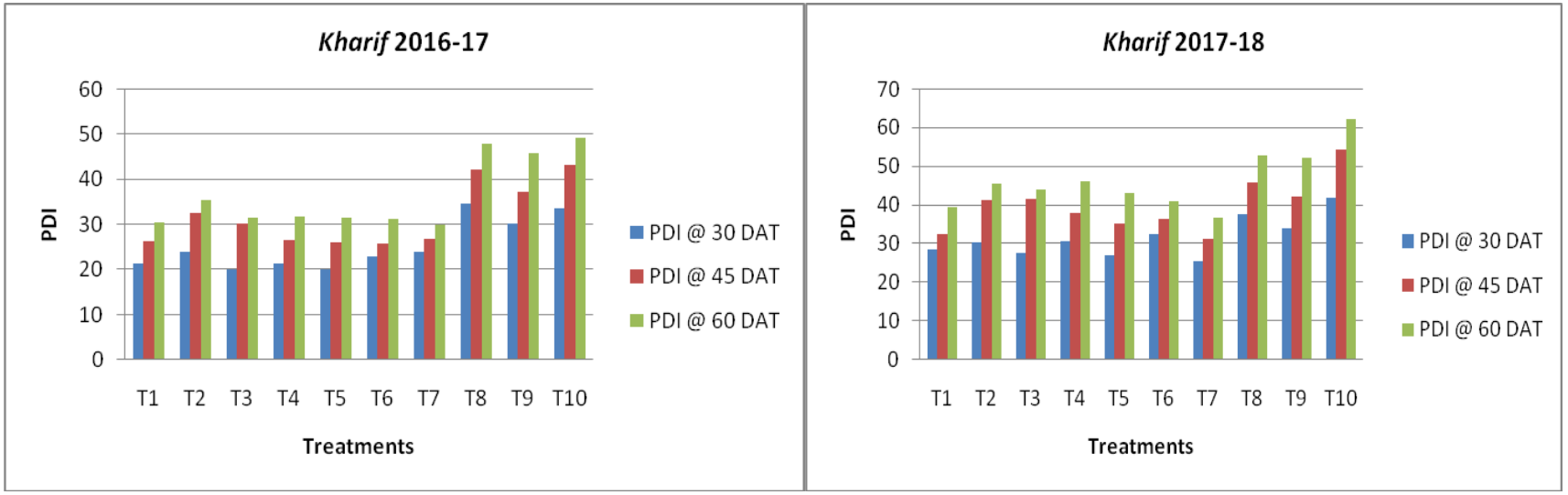
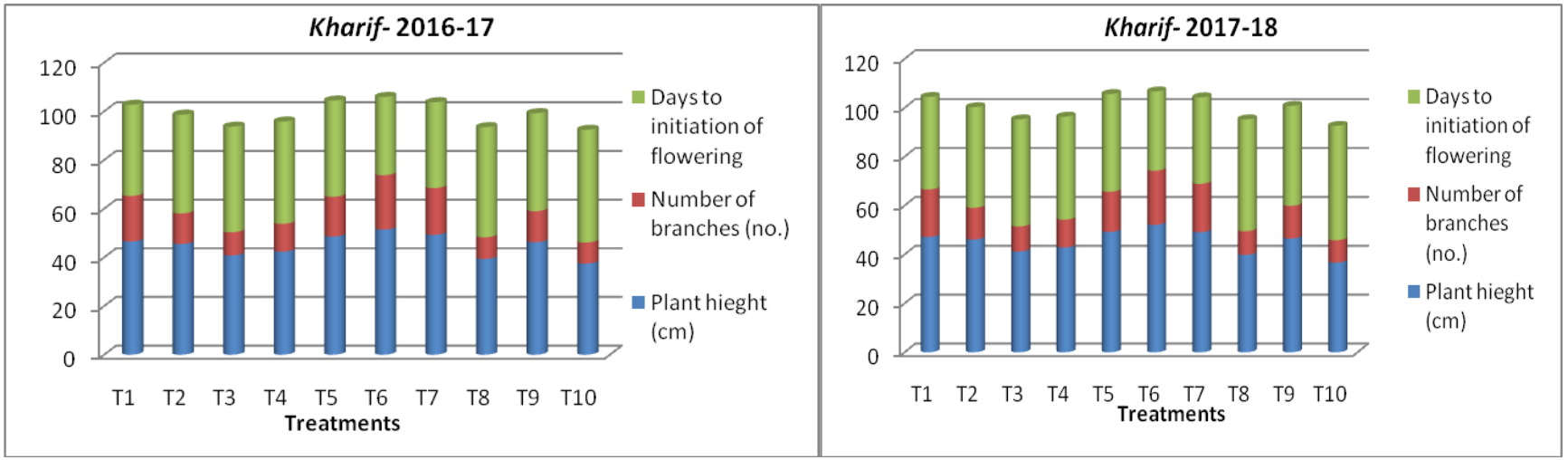


Fig.2 Effect of biotic inducers on growth parameters of chilli during *Kharif* 2016-17 & 2017-18 under field conditions



Similar results were also reported by Noiket *et al.,* (2014), Agbodjato *et al.,* (2015), Jelin*et al.,* (2013), Aleksandrowicz-Trzcinska *et al.,* (2015) respectively in tomato, maize and scots pine sprayed with chitosan and PGPR. The highest plant length, fresh and dry weights were obtained in tomato plants grown from transplants treated with chitosan (Nawar, 2005). Application of chitosan (5%) had the superior effect (El-Tanahy *et al.,* 2012) on all measured vegetative parameters of cowpea plants. Foliar application of chitosan increased common bean plant growth as compared to chitosan untreated plants (Abu-Muriefah, 2013). Besides, chitosan also promoted the growth and yield of tomato (Ibrahim *et al.,* 2015).

In the present study enhanced growth was observed in the plants treated with *P. fluorescens*. Application of PGPR has promoted plant growth by facilitating resource acquisition, modulating plant hormone levels and decreasing the inhibitory effects of various pathogens as biocontrol agents (Glick, 2012).

Plant yield promotion by different biotic inducers

Analysis on yield components showed that, number of fruits per plant, individual fruit weight, fresh weight and dry weight of chilli was influenced by several treatments (Table 4). Application of chitosan (0.1%), *K. alvarezii* (0.4%) and *P. fluorescens* (0.6%) significantly improved plant yield compared to control under field condition during *Kharif*2016-17 *and Kharif*2017-18.

Data from the table 4 elucidated that, chitosan treated chilli plants was found effective significantly by producing highest yield of 1.47 and 1.29 t.ha^{-1} during *Kharif*2016-17 *and Kharif* 2017-18 compared to untreated control (1.19 and 0.97 t.ha^{-1}). The similar trend was also observed in the plants sprayed with *K. alvarezii* and *P. fluorescens* by recording dry weight of 1.42 and 1.24 t.ha^{-1} and 1.40 and 1.19 t.ha^{-1} respectively. Chitosan spray significantly increased number of fruits per plant (192.70 and 193.04), individual fruit weight (2.56 and 2.62 g), fresh weight (4.89 and 4.89 t.ha^{-1}), dry weight (1.47 and 1.47) of chilli compared with the control respectively during *Kharif* 2016-17 *and Kharif*2017-18.

These results are in agreement with the report made by El- Mougy *et al.,* (2006), chitosan treated tomato plants showed increased yield by 66.7 per cent compared with untreated plants. Additionally, El-Tanahy *et al.,* (2012) pointed out that the best yield of cowpea plants were obtained by using chitosan (1213.89 g.plot^{-1}). Foliar application of tomatoes with extract of brown seaweed, *Ascophyllum nodosum* (0.5%) (ANE) reduced disease incidence of *Alternaria solani* and *Xanthomonas campestris* pv *vesicatoria* upto 63 and 44 per cent respectively with increased fruit yield upto 42 per cent compared to controls (Ali *et al.,* 2016).

Similarly, Venkatesh (2016) found increased mean yield of gherkins by 13.33 t.ha^{-1}and 12.17 t.ha^{-1} in gherkin plants sprayed with seaweed extracts *K. alvarezii-1* (0.4%) and *Halymenia durvillae* (1%) respectively in *Kharif* and *rabi* 2016 field experiments. Mishra *et al.,* (2014) reported that, the tomato plants treated with the chitosan based formulation of *Pseudomonas* sp.(206(4) +B-15+ JK-16) recorded the highest activity of ISR against ToLCV and recorded maximum plant height (52.5 ±1.38 cm), total biomass (0.043 ±0.041 kg.plant^{-1}), chlorophyll content (35.2 ±1.02 SPAD), fruit number (26.6 ±0.81) and yield (1.77±0.0.07 kg.plant^{-1}) over the diseased control (0.45 ± 0.01 kg.plant^{-1}).The efficacy of *K. Alvarezii*-1(0.4%) against papaya ringspot virus (PRSV) was tested under field condition by Vijayalakshmi and

Nagaraju (2017), where *K.alvarezii*-1has recorded 71.66 per cent disease control with the yield of 41.87 kg.plant^{-1} compared to untreated control which showed 100 per cent disease incidence.

Pooled analysis of data obtained from both the seasons *Kharif*2016-17 *and Kharif*2017-18 also confirmed the effectiveness of chitosan (0.1%), *K. Alvarezii* (0.4%), Synthetic nucleoside (0.25%) and *P. fluorescens* (0.6%) in reducing the CMV disease incidence by increasing the growth and yield parameters (Table 5). Among them, Chitosan and *K. Alvarezii* showed significantly increased yield of about 1.38 and 1.33 t.ha^{-1} respectively followed by *P. fluorescens* (1.30 t.ha^{-1}) and Synthetic nucleoside (1.28 t.ha^{-1}).

It is concluded in the present study, the increased chilli yield was observed due to reduction in CMV disease incidence with enhanced growth influenced by foliar application of Chitosan, *K. alvarezii* and *P. fluorescens.* Therefore it is possible to recommend above treatments to manage CMV infection effectively under field conditions with enhanced growth and yield of chilli.

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